electrostatically stabilized gel. The rigidity of the gel, indicative of the interaction strength and range, can be quantified by measurement of the elastic modulus G′. We obtain G′ by optically trapping and oscillating a polystyrene particle in the gel and measuring the amplitude and phase of its displacement. From the screened Debye-Hückel potential, G′ is related to an *effective* particle charge Z*, as described by Alexander et al. (1984). Thus we determine Z* as a function of Z_0. The particle charge Z in the presence of interactions can be calculated from G′ by numerical integration of the Poisson-Boltzmann equation (Alexander et al., 1984). In deionized solutions, because there is 1 dissociated H^+ for each particle charge, higher Z* is accompanied by more H^+ and more screening. At low Z*, the interaction is dominated by the particle charge, and G′ increases as Z^{*3}. At high Z*, the interaction is dominated by H^+ screening, and G′ decreases as $\exp(-Z^{*1/2})$. We demonstrate this effect experimentally and determine Z* at G'_{max} for several liposome concentrations. A G′ maximum can still exist in the presence of low ionic-strength salt when $[H^+]$ is comparable to the concentration of salt counterions. Our results show that the elastic moduli of electrostatically stabilized colloidal suspensions in low ionic-strength media are maximal at intermediate values of the particle charge.

408-Pos Board B177
Quantum and All-Atom Molecular Dynamics Simulations of Protonation and Divalent Ion Binding to Phosphatidylinositol 4,5-Bisphosphate (PIP2)
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Phosphatidylinositol 4,5-bisphosphate (PIP2) is a minor component of the inner plasma membrane that is capable of binding to hundreds of intracellular proteins. It carries a large negative charge, has a big lateral surface area, and can form clusters under certain ionic conditions in vitro. We have completed an analysis of the structure of PIP2 at the quantum level and the propensity for PIP2 to bind physiologically relevant divalent cations. We performed a geometry optimization at the Hartree-Fock 6-31G(d) level of theory in vacuum and with a polarized continuum dielectric to determine the conformation of the phospholipid headgroup in the presence of water and its partial charge distribution. The angle between the headgroup and acyl chains is approximately 89 degrees, indicating that the inositol ring may lie flat along the surface of the inner plasma membrane. Next, we employed hybrid quantum mechanics/molecular mechanics (QM/MM) simulations to investigate the protonation state of PIP2 and its interactions with divalent cations such as magnesium or calcium. We test the hypothesis that binding of magnesium to PIP2 is mediated by a water molecule that is absent when calcium binds. We observe that binding of calcium is able to deprotonate PIP2 at one phosphate group, which causes the molecule to decrease in size, and this does not occur when magnesium binds. These results may explain the ability of calcium to induce the formation of PIP2 clusters and phase separation from other phospholipids.

409-Pos Board B178
Membrane Charging and Interfacial Hydration
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Due to thermal motion and molecular polarizability, physical interactions at the membrane-water interface have a pronounced dynamic character. In particular, the interplay between molecular disorder (entropy) and molecular interactions can have unexpected consequences primarily with regard to membrane electrostatics [1,2]. We show experimentally that significant charging occurs for lipid membranes in the presence of highly polarizable solutes such as anions and zwitterionic pH buffers. To complicate matters, this charging process takes place while there is a net deficit of solutes in the immediate vicinity of membranes. The important consequence is that electrostatic forces between macromolecular surfaces are then less screened and can act over long distances. We quantify both membrane electrostatics and solute deficit by using a number of experimental methods including small-angle x-ray scattering, buoyancy measurements, and molecular drift in electric fields. It is also shown that there are solute mixtures in which electrostatic cancellation occurs. These aspects of membrane electrostatics are relevant to studies of membrane fusion and of protein-lipid membrane interactions.
[1] Petrache *et al.* J. Am. Chem. Soc. 2005, [2] Koerner *et al*. Biophys. J. 2011.

410-Pos Board B179
Intramolecular Hydrogen Bonds in Cardiolipin
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Cardiolipin was first isolated in 1941, and has since been implicated in several critical physiological roles. The distribution of cardiolipin species in tissues across prokaryotes and eukaryotes, and its concentration in membranes across which proton gradients drive energy production, is well characterized. The various functions attributed to cardiolipin include the structural stabilization of oxidative phosphorylation complexes in the inner mitochondrial membrane, where it constitutes about 25% of lipid. Its acid-base titration profile suggests a variable head group pKa in the physiological range, which leads to the hypothesis that cardiolipin also serves as a proton reservoir for energy-related protein complexes in the mitochondrial inter membrane space, thylakoid lumen, and at the outer leaflet of bacterial membranes. The importance of cardiolipin in these ascribed roles is further underscored by its involvement in diseases such as Barth's syndrome, where mitochondrial dysfunction is a major factor.
In contrast to this physiological importance, Cardiolipin remains one of the least well characterised lipids from a biophysical perspective. In particular, there is still no consensus on whether it is a hydrogen bond between a protonated phosphate and the free 2-hydroxyl of the bridging glycerol of cardiolipin that explains its anomalous pKa. Furthermore, static 31P solid-state NMR characterisations of the head group have been difficult to interpret. We use our new Boltzmann-statistics maximum entropy analysis to globally analyse slow magic angle spinning spectra, and explain the uncharacteristic 31P static NMR spectra. Together with differential scanning calorimetry analysis, intriguing evidence emerges for the hydrogen bond hypothesis. We further pursue the hypothesis with additional solid-state NMR 31P{1H} heteronuclear correlations (HETCOR) and rotational-echo double-resonance (REDOR) experiments.

411-Pos Board B180
Water Pockets between Acyl Chains and its Relation to Peptide Insertion in Lipid Membranes
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Presence of water in the membrane structure has been invoked to interpret the insertion of highly hydrophilic aminoacid moieties in pools between acyl chains, described as water pockets. Analysis of the band corresponding to the frequency of vibrational symmetric stretching mode of methylenes and the bands of water below and above the phase transition of different lipids by Fourier transform infrared spectroscopy give strong support to the formation of confined water pockets in between the lipid acyl chains. We present a rational description of the water pockets creation and the influence of the adjacent wall formed at the phase transition by analyzing the changes of FTIR-ATR spectra in the regions corresponding to the CH2 and water bands shifts with temperature. The presence of water in these packing defects gives support to the creation of regions with free energy excess due to reinforcement of the water structure. This high energy defects explains the insertion of peptides and aminoacid residues and translocation of peptides through the biomembrane.

412-Pos Board B181
Volumetric Stability of Lipid Bilayers
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The gel phase of DPPC has been the best characterized lipid bilayer. It has therefore been alarming that recent publications have reported a gradual decrease in lipid molecular volume of DPPC multilamellar vesicle dispersions in the gel phase upon repeated thermal cycling between 10°C and 50°C using a commercial densimeter. The considerable size of this decrease would have significant implications for the physical chemistry of biomembranes. We have confirmed this phenomenon with the same densimeter model. By contrast, neutral buoyancy measurements performed with similar thermal cycling show no gradual change in lipid volume in the gel phase at 20°C. Remixing the lipid in the densimeter shows that the apparent volume decrease is an artifact. We conclude that volumes obtained by neutral buoyancy measurements remain accurate and that gel phase DPPC bilayers exist in a volumetrically stable state.
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413-Pos Board B182
Interactions of a Monofluorinated Phospholipid with Saturated Phosphatidylcholines of Different Chain Lengths
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This study uses differential scanning calorimetry (DSC) and fluorescence spectroscopy to study the thermodynamic and kinetic effects of the monofluorinated

phospholipid 1-palmitoyl-2-[16-fluoropalmitoyl]-phosphatidylcholine (F-DPPC) on bilayers composed of the fully saturated phosphatidylcholines 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). In both bilayer systems, DSC thermograms indicate a disappearance of pretransition peaks (T_p) along with a rise in main transition (T_m) hysteresis at elevated F-DPPC mol%. Fluorescence intensity measurements reveal an inverse relationship between F-DPPC mol% and the emission intensity of the environment-sensitive probe 1,6-diphenyl-1,3,5-hexatriene (DPH) below the main transitions of the respective lipids. These trends suggest a growth in interdigitated domains with the incorporation of additional F-DPPC into the bilayer. Significant drops in intensity values were observed at lower F-DPPC mole percentages in the DSPC system than the DMPC system, indicating that the latter lipid has a higher threshold for F-DPPC-induced interdigitation. Our results support that F-DPPC encourages the interdigitated phase (L_βI) in saturated bilayers and highlight the stabilizing effect that long acyl chains have on this phase.

414-Pos Board B183
Not All Hybrid Lipids are Linactants
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Hybrid lipids are thought to be able to perform the function of linactants at membrane domain interfaces: They can reduce line tension and stabilize nanoscopic lipid raft domains in biomembranes. Hybrid lipids are lipids with one saturated chain and one unsaturated chain. Here we provide evidences that only certain hybrid lipids behave like linactants. In this study, we compared three hybrid lipids (i.e., 16:0-181PC (POPC), 16:0-18:2PC, and 16:0-22:4PC) in their abilities to reduce lipid domain size and shift phase boundary. The Lo-Ld phase boundaries of hybrid-lipid/di18:0PC(DSPC)/cholesterol systems were determined from giant unilamellar vesicles (GUV) using fluorescence microscopy. We found that 16:0-22:4PC behaves similarly to a fluid-phase lipid: The Lo and Ld lipid domains in 16:0-22:4PC/DSPC/CHOL mixtures are macroscopic and the phase coexisting region is very wide. On the other hand, 16:0-18:2PC/DSPC/CHOL system has a much narrower Lo-Ld phase coexisting region; however, the lipid domains are still macroscopic. Only POPC/DSPC/CHOL system contains nanoscopic lipid domains. These results were compared with Monte Carlo simulations. Based on the magnitudes of interaction energies, it appears that only mono-unsaturated hybrid lipids behave like linactants, while poly-unsaturated hybrid lipids behave more or less like fluid-phase lipids.

415-Pos Board B184
Elasticity of the Lipid Bilayer Edge. Trends in Line Tension
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The line tension or excess free energy per unit length of a bilayer edge is an essential measure of the toughness of a lipid bilayer and its ability to support nanoscopic pores. Well-converged values for the line tension of model pure lipid bilayer edges are important for evaluation of the tendency of additives to stabilize or destabilize the edge. Experimental measurements of bilayer edge line tension are challenging, and new approaches are still under development. In this study we report trends for line tensions and microscopic details of the lipid bilayer edge from a series of atomistic simulations of phosphatidylcholine lipids with varying degree of saturation and tail lengths. The simulation line tensions we obtain are higher than those reported from experiments. The choice of force-field on the resulting ribbon properties was investigated and found to not affect the results. The energetics of edge formation as an area expansion perturbation to the bilayer state was also explored and explains the simulation line tensions within a factor of two.

416-Pos Board B185
Image Analysis of Phase Separated Langmuir Monolayers Containing Polyunsaturated Fatty Acids
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Results from epifluorescence microscopy studies and image analysis of phase separated Langmuir monolayers of ternary mixtures containing polyunsaturated fatty acids (PUFAs), sphingomyelin, and cholesterol will be presented. Experiments were done using a Langmuir trough and inverted microscope under a sealed chamber providing an inert atmosphere. We will focus on the results and implications from measurements of domain size distribution and area fraction for four different mixed acyl phospholipid species with varying degrees of unsaturation in the acyl chain (1, 2, 4, 6). We have applied a recently developed technique to measure the line tension of these systems using the size distribution [1]. This experimental approach allows us to investigate the relationship between the miscibility phase transition, line tension, and degree of unsaturation even for systems with small domains not otherwise amenable to line tension studies. Experiments described above were combined with more traditional Langmuir film-balance techniques including pressure-area isotherms.
[1] Lee et at., Relating Domain Size Distribution to Line Tension and Molecular Dipole Density in Model Cytoplasmic Myelin Lipid Monolayers. PNAS 108, 9425-9430.

417-Pos Board B186
Effects of Oleic Acid on Stratum Corneum Lipids in Langmuir Monolayers
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The mechanism of oleic acid (OA) as a transdermal permeation enhancer has long been debated. In this study, the interaction between OA and stratum corneum (SC) lipids was investigated with an aqueous monolayer of model SC lipids. Different amount of OA was cospread with equal molar mixture of ceramide, cholesterol and palmitic acid at the air/water interface. The monolayer phase behavior was monitored through surface pressure-molecular area isotherms (π-A isotherms). With increasing OA concentration in the monolayer, the resultant films became more fluid and more compressible. OA also modified the domain structure in SC monolayers as visilized through Brewster Angle Microscope (BAM). The miscibility curve derived from π-A isotherms demonstrated the preferential interaction between OA and SC lipids. IRRAS measurements showed that OA mixed with ceramide and disordered its acyl chains. The acyl chain order of palmitic acid was also lowered by OA but to a lesser extent.

418-Pos Board B187
Comparison of Cholesterol and 25-Hydroxycholesterol in Phase Separated Phospholipid Monolayers
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Experimental studies of the phase separation of coexisting liquid phases in mixed phospholipid/sterol monolayer systems have contributed significantly to our understanding of the unique role that cholesterol plays within lipid membranes. Cholesterol is not unique in its ability to promote phase separation in these model systems. Several cholesterol analogs display similar liquid-liquid phase coexistence in monolayer and bilayer systems. One particularly interesting example of this is 25-hydroxycholesterol (25OH), which has been previously noted to have a kink in its monolayer pressure-area isotherm corresponding to the miscibility phase transition as well as for its pathological effect on the plasma cell membrane. We present the results of experiments using traditional Langmuir film-balance techniques (pressure-area isotherms) and surface potential measurements to identify changes in molecular orientation during monolayer compression. Fluorescence microscopy experiments complement these studies with comparisons of domain size distributions, area fraction, and line tension measurements. From our preliminary work it is clear that there are many similarities between the phase behavior of these two systems as well as many significant differences.

419-Pos Board B188
Physical Properties of an Asymmetric Nanobio Lipid Membrane
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Presence of additional phospholipids in one of alive cell's lipid membrane monolayers is one of the interesting phenomena. A very important part of living cells of biological systems is lipid membrane, and the mechanical properties of this membrane plays an important role in biophysical investigations. It is interesting to evaluate the effect of additional phospholipids insertion in one leaflet of a bilayer on the physical properties of obtained asymmetric lipid membrane. In the present work a coarse-grained molecular dynamics simulation is carried out to compute the physical properties of each leaflet of such a bilayer. Our simulations reveal that the insertion of additional phospholipids into one monolayer results in an asymmetrical change in the lateral pressure of the individual bilayer leaflets. The relative variation in the lateral pressure of the two leaflets as a result of a change in the contribution of the various intermolecular forces may potentially be expressed morphologically.

420-Pos Board B189
Interactions of Cymal-6 and Lipid Vesicles
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The solubilization of biological or liposomal membranes, induced by detergents and detergent-like biomolecules, is important to many technical applications and biological phenomena. In fact, the interactions of classical detergents